

## Ecdysis in a Teleostean Fish, *Agriopus*.

By

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With 6 Text-figures.

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*Agriopus* is a well-known fish of the Southern Hemisphere, and is recorded from South America, South Africa and Australia. On account of its striking appearance it attracted the attention of early travellers, and is first mentioned by Block under the name of *Coryphæna torva*. It has a mane-like dorsal fin and a long vertical forehead, from which it derives its local name "Horse-fish." A further characteristic, mentioned by all observers, is its soft, thick skin devoid of scales. This was specially noted by Cuvier and Valenciennes, who describe the skin of *A. torvus* as "épaisse et lisse comme un cuir bien préparé, sans aucune apparence d'écailles." They add that, among the specimens procured, there were some in which the skin was quite brown and raised in small wart-like prominences, so well marked as to suggest that these individuals represented a different species, though otherwise not dissimilar.

It may further be observed that among such brown specimens there are occasionally some in which the skin has apparently become detached, and hangs in ragged patches from the body. This might readily be put down to imperfect preservation, and consequent detachment of the epidermis, but the same condition was observed in specimens kept alive, and in another species, *A. spinifer*, kept alive for

about two years in an aquarium tank, the skin was observed to become detached over the whole body and fins, leaving a brightly coloured new skin underneath.<sup>1</sup>

In order to obtain further information on this peculiar occurrence, which looks like a process of ecdysis, sections were made of the skin, and these revealed a condition which seems to be of more interest than a process of simple ecdysis, though this, so far as I am aware, is not known to occur in any fish.

The three Cape species (*A. torvus*, *A. spinifer* and *A. verrucosus*) were examined, and, though the process of casting the skin differs in these in some respects, the essential features are the same.

#### *Agriopus torvus*, Cuv. & Val.

In some individuals the skin has a bright, fresh appearance, with clearly defined broad bands of pigment along the body at intervals. In others the whole outer surface of the body and fins is of a dirty brown colour as if coated with a thick layer of diatomaceous growth. This layer may be of considerable thickness.

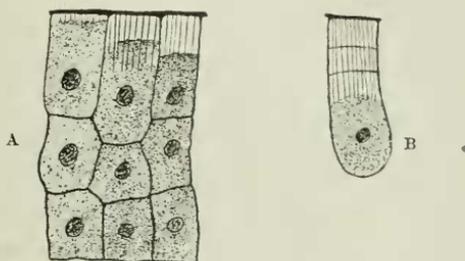
Sections of the brightly coloured skin (Text-figs. 1A and 1B), in which this layer does not appear to be present, show that it is there, but at an early stage of development. The normal elements found in the skin of fishes are present, there being a comparatively thin epidermal layer of about three to five cells in thickness. The lower cells are square, and rest on a well-marked basement membrane. The cells above this layer are rounded or polygonal in form, and are arranged more or less regularly in rows perpendicular to the epithelial surface. The outlines of all these cells are clearly defined. At the base of the epidermis there is a layer of black chromatophores, and scattered chromatophores are sometimes found in the epidermis itself. Sections of the skin in some parts of the body also show numerous saccular mucous

<sup>1</sup> 'Mar. Biol. Report, Union of South Africa,' 1914, p. 102.

glands within the epidermal tissue. The dermis underlying these elements is very thick and tough in this species of *Agriopus*, and there are no scales.

The chief point of interest is the character of the outer cells, and the peculiar substance they produce at their free surface. The earliest stages of this substance can be seen in a fish in which the skin has been partly cast off, leaving certain parts of the body with the clear new skin exposed. The formation of this tissue must be very rapid, for already it is well marked in some places in the new skin, though at

TEXT-FIGS. 1A AND 1B.



Section of skin of *Agriopus torvus* with little or no columnar development. [The degree of magnification is shown by the scale, representing  $10\ \mu$ , accompanying the figures. All figures are drawn by camera lucida, except fig. 6; fig. 2 with Zeiss C objective, the others with  $\frac{1}{12}$  oil-immersion objective.]

other parts there is little or no trace of it. For the purpose of ascertaining the minute structure of the outer cells and the substance they produce, very thin sections ( $2\ \mu$ ) were made, and stained in hæmalum—a stain which was found to be most suitable for the purpose of showing the structure of the cells and their cuticle-like product.

In such sections (Text-fig. 1A) there is a fairly thick outer dark margin, which is continued down into the intercellular substance of the superficial epidermal cells. In a few cases the outer border of the cell was applied to this margin; in most, however, the cytoplasm was separated from the outer border, though still connected to it by fine

fibrils. The space between the outer border and the cell was occupied by a clear substance, in which only the fibrils could be distinguished, and which was sharply marked off from the cytoplasm of the cell.

A later stage is evidently indicated by a cell which is further removed from the surface (Text-fig. 1A). Here the fine fibrils from the outer border are again seen. In some cases they appeared to end abruptly at the cell, but in others they were continued down into the cytoplasm, almost to the nucleus, giving the distal portion of the cell a marked striated appearance.

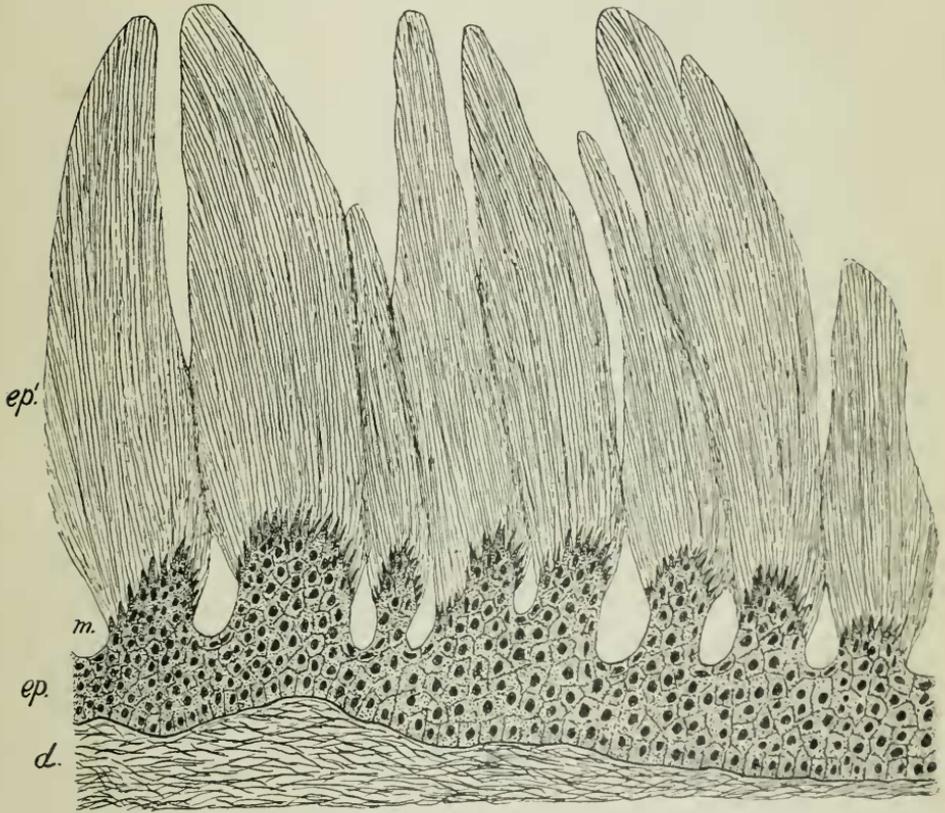
Such stages were found alongside of each other, and at other places (Text-fig. 1B) cells were found still further removed from the surface, so that the space between the outer border and the cell assumed the appearance of a clear short column traversed by longitudinal striations or fibres. Running across this column were what appeared to be transverse septa, not clearly defined and slightly curved, with the concave side toward the cell. The transverse septa varied in number from one to four according to the length of the column. They were not placed at regular intervals from each other (vide Text-fig. 3).

The wall or border between the columns is well marked, of a dark colour, and more clearly defined than the transverse septa. In some cases this wall was seen in such longitudinal sections to be broken up into short dark transverse streaks, which were to be seen more distinctly in transverse sections of the column (Text-fig. 5). The substance of the column in these sections was well marked off from the protoplasm of the cell, except in cases where the fibres penetrated its substance.

If sections of the skin be now examined in which the columns are further developed (Text-fig. 2), and can be seen macroscopically as the thick dark outer layer already mentioned, it is observed that this condition is brought about by the great development of the columns, accompanied by a modification in the outer epithelial cells. These are now

more numerous, and are no longer arranged in rows perpendicular to the surface. There is a great variety in the characters of the outer cells; in some cases they are low and

TEXT-FIG. 2.



Section of skin of *Agriopus torvus* with fully-developed columnar layer. *ep.*, epithelium; *ep'*., columns; *d.*, dermis; *m.*, mucns.

even flattened, as are also their nuclei, in a direction parallel to the epidermis; at other places they are narrow and greatly elongate in a direction at right angles to the epidermis (Text-fig. 3), being drawn out in long finger-like processes, which sometimes attain a length equal to the depth of the

whole epidermis below them. The mode of termination of these long cells varies considerably. They sometimes pass without any clear line of demarcation into the substance of the column above them. At other times this demarcation is well marked, and the striæ of the columns are clearly continued down into their substance. At other places they are not only sharply marked off from the columns, but withdrawn from them, being separated by a well-marked space; in such cases the free end of the cell was often observed to present a vacuolated appearance. In some cases the columnar layer is entirely separated from the cells, and the process of ecdysis is apparently completed. The nuclei in these elongate cells show, in some cases, signs of disintegration. They appear sometimes as broken-up separate masses of chromatin, may assume a half-moon-like appearance, or appear like an irregular cross (Text-fig. 4).

The elongate and flattened cells occurred in groups alternating with each other, the former giving rise to a fan-shaped structure, between which occurred depressions formed by the flattened cells. These depressions assumed the form of rounded spaces filled with homogeneous mucus (Text-fig. 2, *m.*).

The consequence of this arrangement is that the outer columnar tissue is divided up into groups or bundles, which may appear as prominences on the outer surface of the skin. The columns composing these bundles are now greatly elongate. They are divided up by numerous transverse septa, but apparently are not otherwise interrupted, as some could be traced from the outer surface down to the corresponding epithelial cell. Very often of course the section does not pass through the length of the columns as shown in Text-fig. 2, but cuts them transversely or obliquely, when they appear as a number of rounded or oval areas.

Transverse sections of the columns (Text-fig. 5) showed that they vary considerably in diameter in the same section (2-7  $\mu$ ), and that they are on an average smaller in diameter towards the epithelium than near their outer extremities—

a fact which is doubtlessly associated with the narrower character and increased number of the superficial epithelial

TEXT-FIGS. 3, 4, 5, 6.

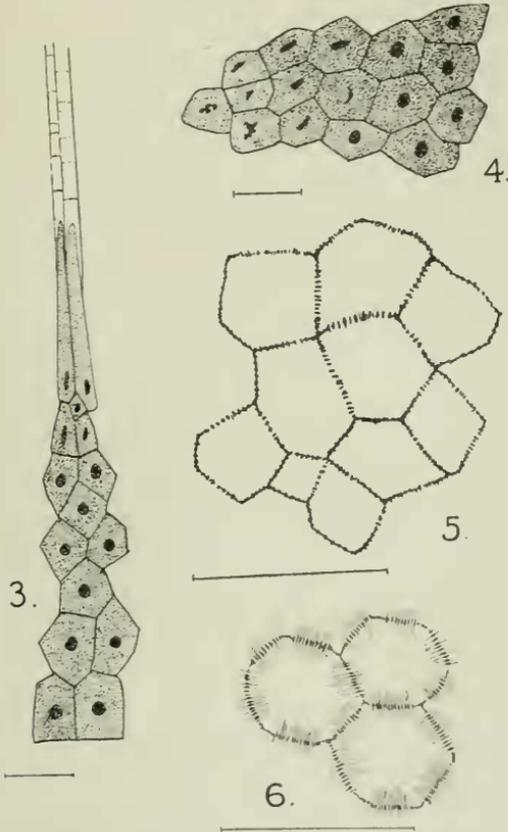


Fig. 3.—Enlarged view of part of the epidermis in above section. Fig. 4.—Oblique section of outer cells of epidermis at a later stage of development showing breaking up of nuclei. Fig. 5.—Transverse section of columns of skin of *Agriopus torvus* showing nature of their walls. Fig. 6.—Optical section of cast skin of *Agriopus spinifer* showing fibrils connecting columns.

cells at this later stage. In one case in which actual measurements were taken, the average diameter of the distal end was  $3\mu$  and of the proximal end  $2.5\mu$ .

The columns are polygonal in outline, so that they present a honey-comb appearance, but are not regular in shape.

In transverse sections further light is thrown on the nature of the walls of the columns. These may be thick or relatively thin, the thick walls being more marked towards the periphery of the bundle of columns. The walls present a dark appearance, being only slightly affected by staining reagents. The walls of the inner columns of the bundles are not so thick, and in thin sections they may be seen at places to consist, not of a single or double homogeneous wall, but of a number of closely-set dark lines, running from one column to the other across the wall, so that the column appears as if held together by slender fibres. Further details were difficult to make out on account of the slightly staining character of the substance and the minute size of the fibre.

How exactly the process of ecdysis is effected was not determined. It appears, in sections of the skin just cast, that the process is merely a pulling off of the columnus, leaving the long, finger-like processes of the epithelial cell projecting. It is probable that this is associated with a breaking down of the superficial layers of epithelial cells, as indicated by the disintegration of their nuclei, which was observed only in these late stages.

#### *Agriopus spinifer*, Günth.

The skin of this species differs from that of *A. torvus* in having scales. These are deeply embedded in the dermis, and are sometimes broad and branched, and at the lateral line apparently fused together. They project as dark needle-like spines, which may or may not penetrate through the epidermis. In the latter case, where they approach the epidermis, they are covered by a proliferation of epidermal cells, presumably ameloblasts. The process of ecdysis is more marked in this species, as the whole of the outer columnar layer is thrown off at one time. This was readily observed in specimens kept alive at the Government Marine Laboratory near Cape Town, and individuals were occasion-

ally got in the trawl with the loose skin only slightly attached to the body. The reason for this is apparently that in this species the active elongate cells of the epidermis are not grouped together in patches, alternating with patches of flattened cells, as in *A. torvus*. The shed skin is not so thick, and is clear and transparent, so that the outlines of the columns can be more distinctly seen. For this reason also the structure of the column, as seen in optical transverse sections, shows more details than in sections of the skin of *A. torvus*. The wall of the column was seen, as in *A. torvus*, to be made up of dark transverse striæ, and these were continued into the interior of the column, where they became very fine and faint in colour. In cases most favourable for observation they were seen to penetrate the column to a depth equal to about one-fourth of its diameter (Text-fig. 6).

#### *Agriopus verrucosus*, Cuv. & Val.

This species is distinguished from the last-named by having no bony projection in front of the eye. The skin is raised into rough projections, arising from the dermis. The structure of the skin appears to be similar to that of the other two species, but no suitably fixed material was available for examination.

The various species of *Agriopus* obviously require revision, and Jenyns is not sure but that his *A. hispidus*, from the coast of Chile, may not be identical with one of the three Cape species. Another species, *A. alboguttatus*, also from the Chilian coast, is of special interest in this inquiry, as the skin is described as covered with very minute horny tubercles. It is possible that here the columnar layer may have become solidified into such harder structures.

#### Nature and Origin of the Columnar Layer.

In view of these facts the question may now be considered as to the significance of the columnar tissue, which is apparently

produced by the external epithelial cells. Does each column represent an excretion from the cells or a transformed and greatly elongate cell or cells? What is the significance of the longitudinal striation, and of the transverse septa?

That they do not represent mucus or other such excretion from the cell might be concluded from their straight course and well-marked walls, and that they represent transformed cells seems to be rendered certain from the structure of their walls, for both in longitudinal and transverse sections these were seen to consist of fibrils, passing from column to column, as seen in stratified epithelium generally, and also noted in some of the parts of the cellular epithelium of *Agriopus* itself.

With regard to the longitudinal striations, which are so well marked in the columns, it must be concluded from their development that they also represent such intercellular fibres, for they may be traced down into the protoplasmic substance of the cell.

The nature and origin of the transverse septa are not so apparent. They appear as distinct lines running across the column, less clearly defined, much thicker and more widely set apart than the fine fibrils, which pass along the column and into it. They are not so thick, however, as the walls of the column. The longitudinal fibres pass through them without interruption, and the most obvious explanation of their nature is that they represent cell-boundaries, like the walls of the column, and that therefore the interseptal spaces represent transformed epidermal cells. As against this, however, it can hardly be supposed that the sides of the column would be so straight and continuous if they represented a number of transformed cells of the epidermis. The fact also that the longitudinal fibrils or striae can be traced without interruption through the whole length of the column is against this interpretation. Again, the interseptal spaces are very unequal, and in nearly all the cases very much smaller than the epidermal cell (cf. Text-fig. 3). The most probable explanation seems to be that these interseptal spaces represent segments of the

striated end of the epithelial cell which have been successively cut off from it in its rapid growth, and that the septa represent modified fibrils or groups of fibrils.

The substance of the column, apart from the fibrils, is composed of a homogeneous structure, which does not readily stain. In some cases, however, the columns are mere empty spaces or vacuoles, their contents apparently having been removed.

The activity of these outer cells of the epidermis suggests a comparison with the activity of the inner cells—the ameloblasts—in the production of enamel. In mammals, as here, long columns or prisms are formed, separated from each other by distinct lines of demarcation, and crossed at intervals by transverse lines. In both cases these columns are produced from the free end of the epithelial cell. In the case of mammals, however, there is a collection of modified tissue—the stellate reticulum—not apparently represented here, and in this the substance of the enamel prisms is believed to arise and to be secreted by the ameloblasts on their outer side, so as to form the calcified prisms.

This stellate reticulum is absent in the formation of enamel in the case of Teleostean fishes—a subject which has been investigated by Tomes, Mummery and Carter. Tomes ('*Phil. Trans.*,' *xciii*, B, 1900) describes the enamel organ of the Hake as consisting of an outer row of cells and an inner row which are the ameloblasts. In the next stage the ameloblasts have apparently disappeared, and in their place is a reticulated stroma of the full dimensions of the enamel which will ultimately appear. This reticulate stroma has a general appearance of fibrillation in the direction at right angles to the dental surface. The meshes of the reticulum are in section round or elongate. The rounded forms looked as if they might be the nuclei of the ameloblast cells, but it was concluded that they represent sections of rods or tubes. With regard to the fate of the ameloblast Tomes is uncertain. It at first greatly increases in length without losing its identity and its nucleus becomes less conspicuous, but it could not be

made out whether the ameloblast cells go to form the rods and tubes of the stroma, the interstitial material being formed outside or between them, or whether the whole cell breaks up into the reticular tissue. Tomes concludes that the enamel is produced by calcification of the reticulate stroma.

Mummery ('Phil. Trans.,' ccciv, B), in a paper on "The Tubular Enamel of the Sparidæ and Labridæ," apparently accepts Tomes' view of the products of the ameloblasts, but Carter ('Quart. Journ. Micr. Sci.,' vol. 63), from a further study of the enamel organ of the Hake, arrives at the conclusion that some at least of the tubular structures are really the nuclei of the ameloblasts; others may be vacuoles in a "fibrillar cytomitoplasm, whose fibres run fairly parallel with the long axis of the cell."

We are not directly concerned here with the origin of the enamel, whether by a transformation of cells or a secretion from their ends, but only with the fact that the lower cells of the epidermis become elongated, and give rise to a fibrillated substance. These long fibrillated cells seem to be comparable with the long fibrillated cells or columns which arise on the outer surface of the epithelium of *Agriopus*, and the fibrils may in both cases be interpreted as no new structure in epithelial cells, but the ordinary connecting fibrils of stratified epithelium, greatly drawn out with the elongation of the cell. Fibrils running across the column are also seen in *Agriopus*, and it would be instructive if these were also found to occur in the elongate cells of the enamel organ of Teleosts.

#### SUMMARY.

(1) The Teleostean fish *Agriopus* appears to shed its skin at certain times.

(2) This shed skin consists of numerous elongate columns, which are striated longitudinally and are continuous with the outer epidermal cells.

(3) The walls of the column are composed of transverse striations, which are prolonged for some distance into the substance of the column.

(4) The walls of the cells of the epidermis are composed of such fibrils or striæ, and it is concluded that the columns represent a greatly elongate and transformed part of the outer cells.

(5) In the columns are also transverse septa or lines, the nature of which is not evident.

(6) The striated columns are compared with certain features in the enamel prisms of mammals, and with the striated elongate cells of the enamel organ of Teleosts.